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Research Paper

Effects of soil composition and temperature on cassava green mite and variety cyanogens potential

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ABSTRACT

Soil compositions and agro-climatic conditions are reported to influence cassava (*Manihot esculenta* Crantz) variety cyanogens potential. A major herbivore pest that leads to economic yield lose in cassava is cassava green mite (CGM), [1]*Mononychellus progresivus* Doreste. *M. progresivus* collected from the coastal Kenya region was evaluated on three varieties at four sites representing different agro-ecological conditions in Kenya during 2011-2013 production periods. Another evaluation was carried out on nine varieties in a screen house to determine varietal cyanogens potential effect to CGM growth and leaf biomass loss. Highest cyanide levels attracted highest CGM density and led to highest leaf biomass loss. Cassava green mite's densities were significantly higher in the dry low midlands of eastern Kenya than at the cooler upper midlands and humid low coastal lands. Leaf cyanogens potential (HCN) were highest at Embu (UM3) than the other sites. Variety MM990183 led with 32.5 ± 4.8 while MM99005 and Ex-Mariakani had 20.6 ± 1.8 and 20.2 ± 2.3 cyanogens levels, respectively. The least HCN was recorded from Mtwapa site (CL3), of 6.5 ± 4.5, 16.3 and 11.4 mg/kg per leaf, for Ex-Mariakani, MM99005 and MM990183. The effect of temperature on CGM numbers was found positively correlated ($R^2 = 0.6024$) indicating high increase of pest density with temperature increase. A low correlation was observed with leaf HCN to CGM density ($R^2 = 0.1686$), where increase of HCN level demonstrated a strong increase of pest density. A clear strong correlation to CGM density was scored where temperature and rainfall were the combined environmental factors ($R^2 = 0.7656$). Similarly, a strong correlation of rainfall and pH level to CGM density growth was shown ($R^2 = 0.7722$). The information on cassava variety cyanogens potential is important for breeding varieties safe for human consumption and which bear little damage from *M. progresivus* mite pest.

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Key words: Cassava green mite; cyanogens potential; pH level; soil composition; human consumption.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is reported to be tolerant to low moisture and poor soils in most regions of Sub-Sahara Africa (SSA) (Hillocks, 2002; Fermont et al., 2009). Improved genotypes have led impetus for increased yield and utilization

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[1] In a detailed morphological study of the African cassava green mite (CGM), Gutierrez (1987) indicated that there is only one designated species representing CGM, and that was *M. progresivus*. The knob of the male aedeagus is small and slender, unlike that of *M. tanajoa* which is globular. Navajas et al. (1994) confirmed the species of *M. progrsivus* in Benin and Congo on molecular analysis on comparison with *M. tanajoa* (Brazil). Our unpublished molecular and morphological data confirm that in Kenya the species is indeed *M. progresivus* though our earlier work indicated *M. tanajoa*.

of the crop (Jennings and Iglesias, 2002; Westby, 2002; Fermont *et al.,* 2009). With the advent of climate change scenario where some regions are no longer considered potential for legume and cereal crops, cassava production is reported as the food insurance crop in those regions (Ferris et al., 1997; Hillock, 2002). Many agro-industries for cassava utilization have been fronted for animal nutrition and industrial raw materials processing (Gomez, 1991; Quenyh and Cecil, 1996; Padmaja et al., 1994). Root HCN level consideration is a pre-requisite to safety for human consumption of raw cassava as incidences of acute toxicity in the SSA region have been reported (Emmanuel et al., 2012; Iglesias et al., 2002; Balagopalan, 2002). Cassava roots have been reported to have higher HCN content level to the amount in the leaves (Wheatley and Chuzel, 1993). Invertebrate herbivores like mealybugs and phytophagous mites have been reported as being of economic importance and constrain production of cassava (Magevand et al., 1987; Yaninek et al., 1989; Yaninek, 1994; Bellotti, 2002). Classical biological control of spider mites have been reported as widely successful in the humid warm regions of SSA with less success in cooler regions (Hanna et al., 2005: Onzo et al., 2005; Zundel et al., 2007). Similarly spider mite pest resistance or tolerance has been attributed to plant vigor, leaf pubescence and antibiosis mechanism (Bellotti and Byrne, 1979; Kawano and Belloti, 1980).

Cassava cyanogens compounds are mainly the linamarin (85%) with lesser amounts of lotaustralin free HCN glycosides found in both foliage and root content (Iglesias et al., 2002; Alves, 2002). No report has detailed how leaf HCN levels contribute to repellence of herbivore pests. Recent studies have indicated high mortalities of the predatory mite *Phytoseiulus longipes* Evans when fed species *Mononychellus progresivus* (Doreste) which feed on cassava leaf tissue (Gutierrez, 1987; Mutisya et al., 2012).

This study explored influence of cassava variety cyanogens potential to CGM density growth and environmental factors effect to both the pest and variety cyanogens potential.

MATERIALS AND METHODS

Screen house varietal evaluation

Nine cassava varieties from coast (three), eastern (three) and western (three) cassava production regions were planted on plastic pots of 18 cm-length × 18 cm-width and 15 cm-height. Sandy loam soil was used in the pots. Each variety had 12 potted cassava plants making a cluster block (4rows × 3plants) in a screen house. The cuttings were watered after every two days to maintain saturated moisture content of the soil with no fertilizer use. One month after planting when plant height was 32 cm, some 10 motile life stages of cassava green mite (CGM) collected from Mtwapa coast area were introduced on each variety. Five days later monitoring of CGM numbers per leaf was carried out by estimating adult stage by aid of a head loop magnifying (×4) lenses. The estimate number of spider mites per leaf was scored to determine injury level in relation to CGM numbers. The screen house (5 m-length × 3 m-width) conditions were temperature 24.2 ± 3°C and relative humidity 63 ± 5%, with clear glass cover on the top. The experiment was repeated thrice. Determination of leaf biomass loss on cassava varieties were taken from randomly picked five leaves (infested with CGM) from each variety representing damage (D) scores of D.1, D.2, D.3, D.4 and D.5. The choice of the sample leaf position was important because the highest numbers of CGM are found on the upper canopy of cassava plant. The five number of leaves for D: 1-5 were collected in Khaki paper (No 25) bags and taken to the laboratory for weighing. The CGM motiles were brushed from the leaves and cleaned with water. The leaves were let dry in room condition before for 10 min before weighing them to determine biomass weight loss. An electronic weighing balance (Santorius Basic-BA 310s, made in Germany) of three decimal units (000.000 g) was used to weigh the leaves after brittle drying them in oven at 60°C. To get single leaf mean weight (mg) of the sample leaves the total leaf weight was divided by five. To arrive at the dry weight (DW) loss or biomass loss at each damage score, D.1 was taken as the leaf DW with no CGM damage score.

Site establishment and mite density score

Three cassava varieties MM99005, MM990183 and Ex-Mariakani were established in plots measuring 10 × 8 m plots at four sites with different agro-ecological conditions. The site locations were at Kiboko (LM5} representing the lowlands located at 02° 12.872 S, 037° 42.960 E, Katumani (LM4), the midlands located at 01° 34. 858 S, 037° 14.580 E, Embu (UM3) of upper midlands at 00° 31.642 S, 037° 28.971 E and Mtwapa (CL3) of coastal lowlands located at 03° 16.024 S, 040° 02.930 E. Ten plot soil samples were taken from each site at 0-20 cm depth with soil auger for chemical analysis (Table 1).

Climatic data for the period 2011 to 2013 representative of the study sites from the nearest meteorological stations of Embu, Katumani, Kiboko and Mtwapa was collected for analysis of the environmental site conditions (Table 2). Land preparation was done before the rains by ploughing and harrowing with a tractor to obtain a fine seed bed. Cassava cuttings were planted at the onset of the rains in mid October of each preceding year. Cassava green mites' score per leaf (second mature of the apex) was carried out from the fourth month after germination with the help of the same head loop magnification lenses (×4). The estimated number of spider mite densities per leaf was

Table 1. Soil chemical composition at Katumani, Embu, Mtwapa and Kiboko plots in 2011.

Site plot	Macronutrients (g/kg)						Micronutrients (mg/kg)				Soil Texture (%)		
	PH	N	P	K	Ca	Mg	Fe	Cu	Zn	Mn	Sand	Clay	Silt
Katumani	4.7	3.4	0.02	0.9	3.3	0.2	195.0	2.3	3.3	118.7	59	30	11
Mtwapa	4.9	1.9	0.01	0.1	1.7	0.1	57.8	0.1	3.3	80.7	69	30	1
Kiboko	7.4	1.6	0.04	0.2	1.5	0.3	0.2	0.0	0.0	-	61	18	11
Embu	6.7	3.1	0.01	0.5	2.0	0.4	0.2	0.0	0.0	1.64	42	56	2

Table 2. Climatic conditions in the study sites.

Year	Site	Rainfall (mm)	Temp (°C)	RH (%)	Description
2011	Kiboko (LM5)	64.0^{c} + I*	24.8^{a}	82.9^{a}	Hot, dry
	Katumani (LM4)	69.5^{c}	19.5^{b}	64.1^{c}	Warm, dry
	Embu (UM3)	127.0^{a}	17.2^{c}	63.4^{c}	Cool, wet
	Mtwapa (CL3)	106.8^{b}	26.8^{a}	77.3^{b}	Hot, wet
2012	Kiboko (LM5)	42.7^{a} +I*	23.6^{a}	83.9^{a}	Hot, dry
	Katumani (LM4)	41.7^{a}	19.9^{b}	58.6^{c}	Warm, dry
	Embu (UM3)	119.6^{a}	17.7^{c}	63.8^{c}	Cool, wet
	Mtwapa (CL3)	104.7^{b}	26.6^{a}	76.0^{b}	Hot, wet
2013	Kiboko (LM5)	52.6^{c} +I*	25.2^{a}	74.9^{b}	Hot, dry
	Katumani (LM4)	81.2^{b}	22.5^{b}	58.6^{c}	Warm, dry
	Embu (UM3)	131.3^{a}	18.3^{c}	63.8^{c}	Cool, wet
	Mtwapa (CL3)	124.4^{a}	26.3^{a}	76.0^{b}	Hot, wet

*Supplementary irrigation at Kiboko of 960 mm during the production period. Different letters denote significant (P<0.001), SNK at 5% level.

scored ranging from a few individual motiles to over hundred as density level in relation to leaf HCN level and climatic conditions. SAS software Version 8 (2001) was used for analysis of variance of spider mite densities, separating mean values by Student Newman Keuls (SNK) Post Hog Test.

Leaf and root cyanogens analyses

Cassava leaf cyanide (HCN) levels were determined by Picric Acid analysis (Natural Research Institute, 1996) both from the nine screen house varieties and the four field plots. Briefly, fresh cassava leaf and roots were cut into disks of specific diameter of 1 cm and were inserted into the bottom of 1.5 cm diameter tube and 5 drops of Tuluone added to leaf and root disks in the test-tube to vaporize the gaseous cyanide (HCN). A Whatman No 1 (6 × 1 cm) strip paper was soaked in Picric acid solution and inserted in each test-tube cocked at the opening to avoid touching the Tuluone-wet leaf and left for 24 h period for colour change. A control test-tube treatment of leaf and root discs was set along all the variety tubes and replicated three times. The colour chart was from the range of 1-9 starting with yellow (no cyanide) to dark brown of highest cyanide level according to Natural Research Institute (1996) procedure. The reading ranges where calculated for mean HCN values of leaf and root values. Analysis of variance was carried to determine significance difference of cyanogens levels of the different cassava varieties. SAS Version 8 (2001) used for means separation with Student Newman Keuls (S.N.K) Post Hog Test. Linear and multiple regression analyses was done to determine the effects of soil pH on cassava root cyanogens level and CGM density (with Pearson correlation (r) values at 5% level).

RESULTS

Screen house leaf cyanide effect

The varieties with cyanide (HCN) content levels >10<30 mg/kg had the highest spider mite population peaks than those of <10 cyanide levels as shown in Table 3. Variety Kalezo from coastal lowlands region had cyanide content of 20 mg/kg with CGM peak score of 1370 CGM/leaf. Karibuni

Table 3. Mean (±Standard Error) number of CGM /leaf and biomass losses (%) in relation to cyanide (HCN) levels on cassava leaf.

Cassava variety	HCN (mg/kg)	Peak No. CGM	Net biomass (%) loss
Kalezo	20.0 ± 2.2^a	1370 ± 5.2^a	67.2 ± 0.5^a
Karibuni	20.0 ± 2.3^a	1170 ± 2.6^b	64.1 ± 0.4^b
Tajirika	12.5 ± 0.9^b	860 ± 10.2^c	29.4 ± 0.4^d
MM990183	12.5 ± 0.9^b	1050 ± 22.3^b	54.5 ± 0.3^c
MM99005	12.5 ± 0.8^b	1020 ± 0.8^b	55.6 ± 0.3^c
X-Mariakani	20.0 ± 2.3^a	1190 ± 1.6^b	54.4 ± 0.5^c
MM97/3567	8.5 ± 0.9^c	585 ± 1.7^c	-
MM96/2480	20.0 ± 2.3^a	1060 ± 3.3^b	66.3 ± 0.2^a
MM96/9308	14.0 ± 1.8^b	580 ± 0.8^c	46.2 ± 0.3^d

Means with different letters across columns are significantly different ($P<0.001$), S.N.K Test.

variety from the same coastal region was third with mite density peak of 1170 mites /leaf and similarly with HCN of 20 mg/kg. The X-Mariakani variety from eastern lowlands led in third position with the highest peak mite infestations of 1190 CGM/leaf with same HCN content of 20.0 mg/kg. Varieties MM99005 and MM990183 of eastern lowlands had HCN level of 12.5 mg/kg and exhibited mid infestation levels of 1020 and 1050 mites/leaf with biomass loss of 55 and 54%. The three varieties from western midlands had low CGM densities of 585, 580 and 760 for MM97/3567, MM96/9308 and MM96/2480 respectively. These low CGM densities similarly caused low biomass losses.

Mite density and cyanogens level

Cassava green mite (CGM) densities were significantly ($P<0.05$) higher in the dry low midlands (LM4 and LM5) of eastern Kenya than at the other sites of cooler upper midlands (UM3) and humid low coastal lands (CL3) (Table 3). Site comparison indicated that Kiboko (LM5) had the highest number of CGM per leaf where MM990183 had a mean of 125 ± 11, followed by MM99005 and Ex-Mariakani with 121 ± 13 and 118 ± 21, respectively. Embu (UM3) had least CGM densities of 4 ± 2, 6 ± 3 and 3 ± 1 mites / leaf for varieties Ex-Mariakani, MM99005 and MM990183.

Leaf cyanogens potential was significantly ($P<0.05$) higher at Embu (UM3) than in the other sites. Variety MM990183 led with 32.5 ± 4.8 mg/kg while MM99005 and Ex-Mariakani had 20.6 ± 1.8 and 20.2 ± 2.3 mg/kg, respectively (Table 4). Notably, the least cyanogens potential (HCN) was from Mtwapa site (CL3) of 6.5 ± 4.5, 16.3 and 11.4 mg/kg per leaf, for Ex-Mariakani, MM99005 and MM990183. Specific site varietal CGM densities was not significantly difference ($P>0.05$) while cyanogens potential was significantly different ($P<0.001$) among the three field evaluated varieties. Likewise, varietal CGM density correlation (R^2) was low but very strong on cyanogens potential.

Environmental factor effect

The effect of temperature to CGM numbers was found positively correlated ($R^2 = 0.6024$) as shown in Figure 1a, indicating medium increase of pest density with temperature increase. Likewise a positive correlation was observed of leaf cyanogens to CGM density ($R^2 = 0.1686$), where increase of cyanogens level demonstrated an increase of pest density (Figure 1b). The relationship between pH level and root cyanogens potential was weak ($R^2 = 0.14$). Further, the percentage (%) sand composition was cyanogens level was weak ($R^2 = 0.056$). But there was medium strong relationship ($R^2 = 0.3511$) between % clay and root cyanogens potential.

Soil composition and pH effect

Soil pH had little effect to cassava root cyanogens level ($R^2 = 0.069$) as observed that most of the sites had close similar levels (Figure 2a). Likewise percentage (%) soil sand had little effect to root cyanogens potential ($R^2 = -0.0377$) as shown in Figure 2b. Soil clay had medium correlation to root cyanogens of cassava variety ($R^2 = -0.3542$).

Compound factor effect

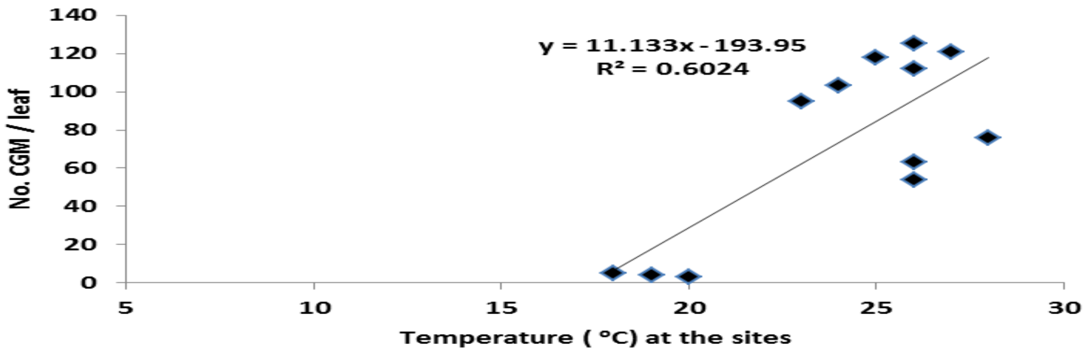
Combined effect of temperature and root cyanogens effected little increase of CGM density ($R^2 = 0. 125$) as shown in Table 5. Similarly, combined soil elements N, P and K had little effect on CGM density growth ($R^2 = 0.279$). Combined effects of rainfall, temperature and cyanogens

Table 4. Cassava green mites' densities (± Standard Error) on three varieties in relation to leaf cyanogens potential (mg/kg) at the sites.

Site / AEZ	Cassava variety	CGM density /leaf	Leaf HCN (mg/kg)
Kiboko (LM5)	Ex-Mariakani	118 ± 1.8^{a}	12.5 ± 0.2^{c}
	MM99005	121 ± 1.1^{a}	16.3 ± 0.4^{b}
	MM990183	125 ± 0.9^{a}	20.0 ± 0.3^{a}
	P (R^2)	*0.771 (0.208)*	*<0.001(0.998)*
Mtwapa (CL3)	Ex-Mariakani	63 ± 1.3^{b}	6.5 ± 0.4^{c}
	MM99005	76 ± 1.5^{a}	16.3 ± 0.4^{a}
	MM990183	54 ± 15^{b}	11.4 ± 0.2^{b}
	P (R^2)	*0.017(0.869)*	*<0.001 (0.991)*
Embu (UM3)	Ex-Mariakani	4 ± 0.2^{a}	20.2 ± 0.2^{b}
	MM99005	6 ± 0.3^{a}	20.6 ± 0.2^{b}
	MM990183	3 ± 0.1^{a}	32.5 ± 0.4^{a}
	P (R^2)	*0.123 (0.879)*	*<0.001 (0.993)*
Katumani (LM4)	Ex-Mariakani	103 ± 2.2^{a}	20.1 ± 0.2^{a}
	MM99005	112 ± 1.2^{a}	16.3 ± 0.2^{b}
	MM990183	95 ± 1.5^{a}	16.5 ± 0.3^{b}
	P (R^2)	*0.606 (0.439)*	*0.002 (0.977)*

Similar letters denote no significance difference (P>0.05) within columns at 5% level.

(a) Relationship between temperature and CGM density



(b) Relationship between leaf cyanogens and CGM density

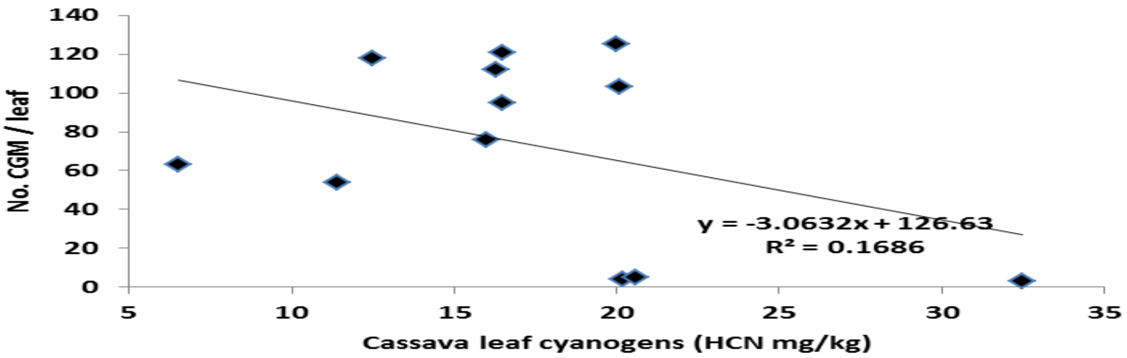
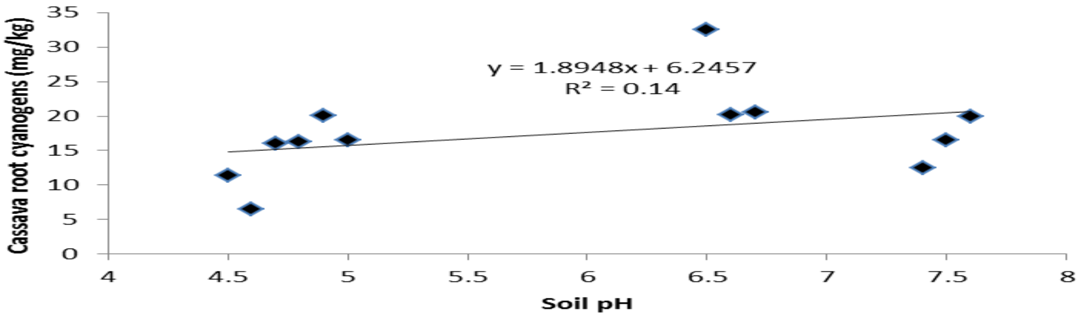
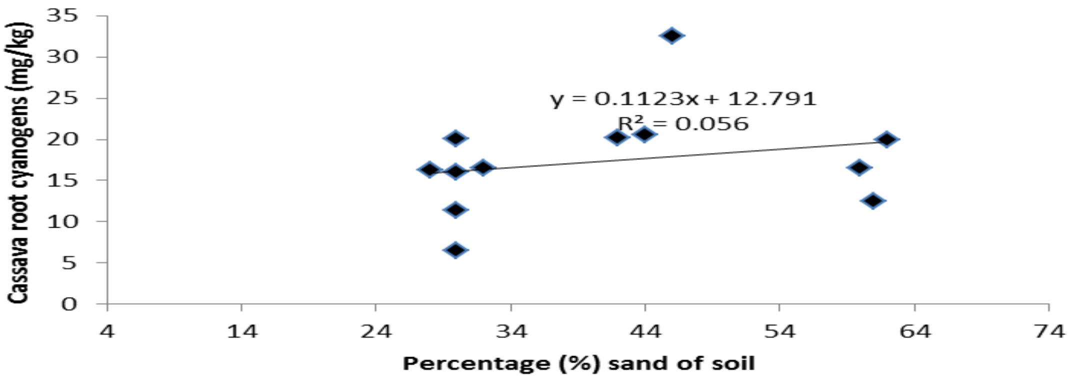


Figure 1. Relationships between (a) temperature and CGM density, (b) cassava leaf cyanogens (HCN) and CGM density.


(a) Relationship between soil pH and root cyanogens


(b) Relationship between % sand and root cyanogens

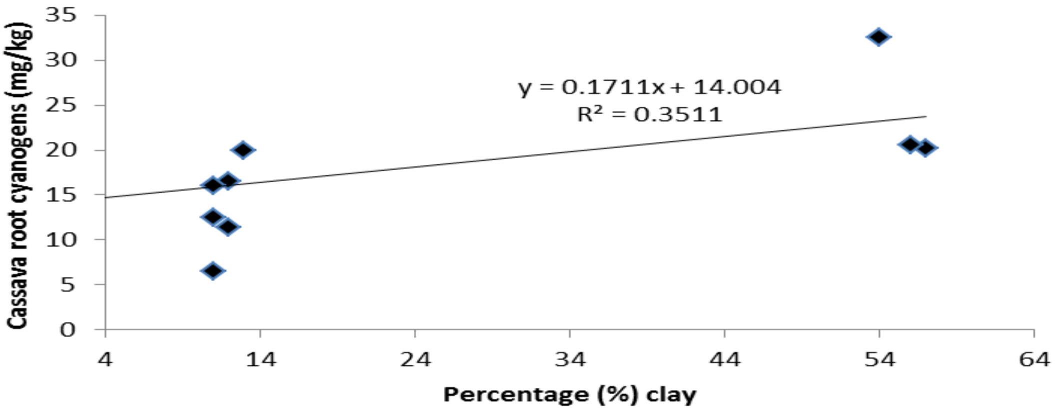

(c) Relationship between % clay and root cyanogens

Figure 2. Relationships between (a) soil pH and cassava root cyanogens, (b) % sand and root cyanogens, (c)% clay and root cyanogens.

had strong effect on CGM density growth (R^2 = 0.7656). Further, a strong positive correlation of rainfall and pH to CGM density growth was determined in the results (R^2 = 0.7722).

DISCUSSION

The different agro-ecological zones in Kenya define cassava green mites (CGM) density level occurrence on cassava varieties, as the present study results have shown. Yaninek et al. (1989) demonstrated that CGM population dynamics were greatly influenced by temperature. The conclusion on the study was that CGM was temperature-dependent for its fast growth. The CGM species (referred to as *M. tanajoa*) in most parts of Africa continue to cause damage on a good number of local and improved cassava varieties even after release of the phytoseiid *Typhloromalus aripo* De Leon close to twenty years ago (Yaninek and Hanna, 2003). Efficient biological control impact has been reported in the warm

Table 5. Relationships of cassava green mite (CGM) densities and physical and edaphic factors.

Element Description	CGM density	R^2	P
Temperature (t), HCN	Y= -26.06+4.27te-0.46hcn	0.125	0.548
N, P, K	Y= 99.47+0.2k-1434.97-6.98n	0.279	0.427
Temperature (t) , HCN, pH	Y=245.6+7.7te-2.8hcn+25.84ph	0.320	0.352
Temperature (t), HCN, rainfall	Y= 35.99+4.49te+0.898hcn-0.98mm	0.453	0.164
Temperature, rainfall (mm)	Y= 107.63 + 2.45t -1.05mm	0.7656	0.002
Rainfall (mm) , Ph	Y= 126.30 – 1.06mm + 5.27ph	0.7222	0.003

humid regions of the Continent of Africa (Kariuki et al., 2005; Zannou et al., 2007; Yaninek and Hanna, 2003).

The nine varieties evaluated in the screen house demonstrated that cyanogens potential was related to genetic potential of a cultivar (Yeoh et al., 1998; Emannuel et al., 2012). Highest leaf HCN cultivar had the highest number of CGM and biomass loss. The next step was determination of environmental factors influence to the overall CGM density growth from different sites of the hot warm (LM5), humid (CL3) and cool (UM3) sites. In the study, it was shown that the highest CGM densities occurred in the drier eastern Kenya region (LM4 and LM5) than the coastal (CL3) and cooler (UM3) regions of the country (Yaninek et al., 1989; Kariuki et al., 2000). This was in agreement with results reported by other ecological workers indicating high mite mortalities where high rainfall amounts are experienced (Boudreaux, 1958; Yaninek et al., 1987; Yaninek et al., 1989). The different sites studied indicated that soil composition was mainly different on texture and major fertility elements of N, P and K among the sites. Alves (2002) reported that cassava is a major miner of soil nutrients especially where little input was added during production period. High nitrate fertilizer input has been cited as a factor of increasing variety cyanogens potential while potassium (K^+) has been reported to reduce cyanide content (Attalla et al., 2001; Yeoh et al., 1998). In a related study, Emmanuel et al. (2012) reported low cyanogens varieties of cassava leading to cassava mosaic disease resistance. All the varieties evaluated in the present study had medium cyanogens potential but higher than FAO/WHO recommended level of <10.0 mg/kg (FAO/WHO, 1991). The study results indicated that the cool site of Embu (UM3) led with the highest variety cyanogens potential but CGM densities remained low due to low temperatures during the production period.

The highest CGM density growth correlation was on temperature while cyanogens increase had medium factor influence to the mite population increase in the optimum temperature conditions. The results led to indication that low cyanide cassava varieties which were suitable for human consumption would have least CGM damage (Yeoh et al., 1998). Considering the fact that several factors in the environment would bear the final variety cyanogens potential of the varieties the compound factors were collectively important for farmers at particular sites of specific edaphic and other climatic conditions (Emmanuel et al., 2012). The major elements of N, P and K effect to CGM density were found weak (R^2 = 0.279). Combined temperature, rainfall amount and variety cyanogens potential had medium positive effect to CGM (R^2 = 0.453). The strongest significant positive effect of environmental factors to CGM density increase was of combined temperature-rainfall amount (of R^2 = 0.7656). As more breeding of high yielding cassava get focused towards attainment of low cyanogens potential the results of the present study point to possible attainment of varieties of less CGM damage if those results are confirmed that CGM prefers high cyanide varieties to low content potential ones (Emmanuel et al., 2012). While site specific varietal CGM density correlation was low among genotypes as a result of temperature difference among other factors, cyanogens potential was significant different among the same varieties. As more studies continue to confirm the true species identity with combined tools of morphological and molecular systematics approach of major concern at present is the development of varieties fairly resistant to CGM damage of which the present study results point to an indicator of low cyanogens content leading to less plant damage (Navajas et al., 1994; Bellotti, 2002).

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